OBSERVATIONS ON THE PROTEOLYTIC ENZYME OF BACILLUS PROTEUS *

STUDIES IN BACTERIAL METABOLISM, XL

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The rôle of enzymes in the manifestations of cellular activity, both in unicellular and multicellular organisms, has become a prominent subject of investigation. Evidence is accumulating which strongly supports the view that cellular function is intimately associated with enzyme action. The problem is inherently a complex one, but certain aspects of it may be advantageously approached through the bacteria. Here the enzymes of a single type of cell may be examined under varied conditions, quite free from complications introduced by heterologous cells. A study of bacterial enzymes, furthermore, should eventually lead to facts of practical importance, for enzymes probably play a not unimportant part in the phenomena of microbic disease.

One of the most important phases of cellular activity is that of nutrition; so prominent is the nutritional phase in the bacteria that it largely overshadows their other activities, and the nature and extent of their metabolism largely determine their function in the economy of Nature.

Bacterial enzymes are conveniently divided into two classes: those which are soluble—exo-enzymes—and those which are insoluble, appearing in solution when the bacterial cell is ruptured.

Among the soluble or exo-enzymes, the proteolytic enzyme produced by Bacillus proteus is notworthy both for its activity and the readiness with which it may be obtained in solution free from bacteria. This enzyme is formed in plain broth and plain gelatin during the growth of the organism. It may be obtained in an active state, free from bacteria, by passing the broth or liquefied gelatin culture through a Berkefeld filter; the enzyme, being in solution, passes through with the filtrate, leaving the bacteria behind. Exposure of cultures of B. proteus to moderate heat, or to the germicidal action of 0.5% carbolic acid, kills the organism but leaves the enzyme practically unimpaired in activity.

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This enzyme and some of the conditions favoring its production have been studied by Auerbach,[1] Berghaus,[2] Kendall,[3] Kendall, Day, and Walker,[4] and others. The essential details may be summarized as follows:

1. The proteolytic enzyme of B. proteus appears in an active state in plain broth and plain gelatin cultures of this organism, from which it may be obtained free from organisms by filtration of the culture through unglazed porcelain.

2. The enzyme is not present in an active state in dextrose broth or dextrose gelatin cultures of B. proteus.

3. Very small amounts of dextrose added to plain broth or plain gelatin delay the appearance of this enzyme.

4. The enzyme, freed from bacteria, will liquefy sterile dextrose gelatin as readily as it will liquefy plain gelatin.

5. Neither moderate amounts of organic acids nor relatively large percentages of dextrose have appreciable influence upon the activity of the bacteria-free enzyme as measured by its gelatin-liquefying power in sterile gelatin.

One of us[3] has advanced a theory in explanation of these various phenomena, which, in the specific case of B. proteus, rests upon well-defined features of its metabolism.

B. proteus, growing in nutrient broth or gelatin containing no utilizable carbohydrates (or other non-nitrogenous substances of similar composition), obtains its food requirements both for structure and energy from the protein constituents of the medium. In similar media containing utilizable carbohydrates as well, the structural needs are, as before, largely obtained from nitrogenous compounds, but the energy requirements are obtained at the expense of the carbohydrate. The proteolytic enzyme cannot be detected in those cultures so long as there is residual sugar. Small amounts of sugar delay the appearance of the enzyme. The production of enzyme in demonstrable form, under these conditions, is invariably associated with a relatively rapid increase in ammonia (deaminization). Deaminization is an index of protein decomposition. This soluble proteolytic enzyme therefore appears to be analogous to the soluble exo-enzymes of the intestinal tract, in so far as it prepares protein for assimilation by the organism.

1. Arch. f. Hyg., 1897, 31, p. 311.
2. Ibid., 1906, 64, p. 1.
3. Boston Med. and Surg. Jour., 1913, 168, p. 825.
4. Jour. Am. Chem. Soc., 1914, 36, p. 1965.

In the intracellular utilization of the products of its activity, however, the enzyme has no part. It is a noteworthy fact that sterile solutions containing the enzyme liquefy sugar gelatin quite as rapidly and extensively as non-sugar-containing gelatin, indicating that utilizable sugars in themselves do not interfere with the activity of the mature enzyme, altho they create conditions unfavorable for its formation.

If utilizable carbohydrate is added to the medium in which B. proteus is to be cultivated, the bacteria theoretically may derive that portion of their nutritive requirement which is to be transferred into energy, either from the nitrogenous protein derivatives or from the non-nitrogenous carbohydrates. That portion of their nutritive requirements which is needed for structural purposes[5] must be obtained chiefly from the nitrogenous elements of the media, bcause the carbohydrates do not contain nitrogen. As a matter of fact, B. proteus invariably obtains the energy requirements from carbohydrates, if such be present in the medium in available form, leaving the protein constituents practically intact. A proteolytic enzyme is not theoretically required for the utilization of carbohydrate and the observations of Auerbach and others, quoted, indicate that none is formed in media containing fermentable sugars.

The experiments here reported were designed to show the effect of dextrose, a readily fermentable sugar, upon the formation and activity of the proteolytic enzyme of B. proteus. For convenience, the order observed in the discussion of the enzyme of B. proteus outlined at the beginning of our paper, has been maintained. The details of the analytic methods employed have been discussed in previous communications,[6] and will not be repeated here.

The organism was grown in plain gelatin, and in plain gelatin of identical composition re-inforced by the addition of 1% dextrose. To facilitate filtration through unglazed porcelain filters, 5% gelatin was used in the entire series of experiments. Ordinarily 10-12% gelatin is used in the preparation of nutrient gelatin.

Table 1 shows the essential analytic details in concrete form. It will be seen that the plain gelatin was completely liquefied within the first twenty-four hours' growth, the reaction became progressively

5. Kendall: Jour. Med. Research, 1911, 24, p. 411; 1912, 25, p. 117. Boston Med. and Surg. Jour., 1911, 164, p. 288; 1913, 168, p. 825.
Kendall and Farmer: Jour. Biol. Chem., 1912, 12, p. 215.
Kendall, Day, and Walker: Jour. Am. Chem. Soc., 1913, 35, p. 1201; 1914, 36, p. 1937 (for full details and analysis).

6. Jour. Am. Chem. Soc., 1913, 35, p. 1201.

TABLE 1

THE INFLUENCE OF DEXTROSE UPON THE PROTEOLYTIC ENZYME OF BACILLUS PROTEUS

Days	Plain Gelatin					One Percent Dextrose Gelatin				
	Reaction			Ammonia mg. per 100 c.c. Gelatin		Reaction			Ammonia mg. per 100 c.c. Gelatin	
	Alizarin	Neutral Red	Phenol-phthalein			Alizarin	Neutral Red	Phenol-phthalein		
1	+0.70	+0.30	+0.50	2.8	*L*	+1.00	+1.90	+1.80	0.7	*S*
4	—0.80	—0.30	+0.90	25.9	*L*	+3.20	+3.00	+2.60	1.4	*S*
9	—1.90	—0.90	+0.10	64.4	*L*	+4.20	+5.80	+5.90	1.4	*S*
16	—7.80	—4.00	—2.10	43.7	*L*	+3.20	+3.60	+3.80	2.1	*S*

+ = acid; — = alkaline. Reaction expressed in c.c. of N/1 acid or alkali to neutralize 100 c.c. of culture above that of the controls. *L* = liquefied; *S* = solid. Ammonia expressed as milligrams N increase per 100 c.c. culture over that of the controls.

alkaline and the deaminization as measured by the increase in ammonia, progressed rapidly. The sterile Berkefeld filtrate of this culture liquefied rapidly, showing the presence of a soluble proteolytic enzyme. These data collectively indicate an active and progressive decomposition of the protein constituents of the medium.

No liquefaction of the dextrose gelatin occurred even after 16 days' incubation, altho there was an abundant growth of the organisms. The reaction became progressively acid and there was practically no increase in ammonia (deaminization),—facts indicating a minimal action upon the protein constituents of the medium, but a decided fermentation of the dextrose. The sterile Berkefeld filtrate of this dextrose gelatin culture was wholly without visible action upon gelatin. No evidence of a soluble proteolytic enzyme was obtained.

TABLE 2

THE INFLUENCE OF DEXTROSE IN PROGRESSIVE AMOUNTS ON THE DEVELOPMENT OF BACILLUS PROTEUS IN PLAIN GELATIN

Days	Sugar-free Gelatin		0.1% Dextrose Gelatin		0.2% Dextrose Gelatin	
	Reaction	Mg. NH_3 per 100 c.c.	Reaction	Mg. NH_3 per 100 c.c.	Reaction	Mg. NH_3 per 100 c.c.
1	—0.20	4.9 *L*	+1.05	*a* 3.5 *L*	+1.40	0.7
2	—0.40	13.3 *L*	+0.75	*a* 8.4 *L*	+1.35	*a* 3.5 *L*
3	—0.50	18.9 *L*	+0.70	*a* 14.0 *L*	+1.00	*a* 7.7 *L*
4	—0.95	49.7 *L*	+0.60	*a* 17.5 *L*	+0.95	*a* 9.1 *L*
5	—1.00	59.5 *L*	—1.50	*a* 34.3 *L*	+0.65	*a* 11.2 *L*
10	—4.65	183.4 *L*	—1.60	*a* 78.4 *L*	+0.60	*a* 35.7 *L*
16	—2.90	130.9 *L*	—1.70	*a* 100.8 *L*	+0.30	*a* 104.3 *L*

L = gelatin liquefied; enzyme in liquefied gelatin; *a* = sugar completely removed.

This experiment shows clearly that the addition of dextrose to gelatin cultures of B. proteus causes a striking difference in the nature and extent of the decomposition of the protein constituents of the medium from that observed when dextrose is absent: The reaction becomes strongly acid when dextrose is present, formation of ammonia (deaminization) is minimal, and the dextrose culture does not become liquefied even after prolonged incubation; there is no evidence of a soluble proteolytic enzyme in the dextrose culture.

An important question presents itself: Does the absence of a soluble proteolytic enzyme in a 1% dextrose gelatin culture of B. proteus indicate an inhibition of the activity of the enzyme by the acid products of fermentation, or does the dextrose actually prevent the formation of the enzyme? If the latter possibility were the one realized,

it would provide experimental evidence of a direct relation between enzyme formation and nutritional stimuli.

One method of obtaining information upon this somewhat complex problem is to add gradually increasing amounts of dextrose to plain gelatin and study the development of the organism with respect to metabolism and enzyme formation in their relation to this sugar. Table 2 summarizes such an experiment. Several important facts are clearly set forth therein:

1. In plain gelatin the evidences of proteolytic activity are unmistakable. The reaction, even in 24 hours, is alkaline; some deaminization has taken place; and the medium is completely liquefied. A sterile Berkefeld filtrate of this sugar-free culture will liquefy gelatin, showing that B. proteus has formed a soluble proteolytic enzyme in the medium.

TABLE 2—*Continued*

THE INFLUENCE OF DEXTROSE IN PROGRESSIVE AMOUNTS ON THE DEVELOPMENT OF BACILLUS PROTEUS IN PLAIN GELATIN

0.3% Dextrose Gelatin		0.4% Dextrose Gelatin		0.5% Dextrose Gelatin		1.0% Dextrose Gelatin	
Reaction	Mg. NH_3 per 100 c.c.	Reaction	Mg. NH_3 per 100 c.c.	Reaction	Mg. NH_3 per 100 c.c.	Reaction	Mg. NH_3 per 100 c.c.
+2.10	1.4	+2.75	0	+2.70	0.7	+2.80	0
+1.60	2.8	+2.30	0.7	+3.10	0.7	+3.10	0.7
+1.50	*a* 4.9 *L*	+2.25	2.1	+3.40	1.4	+3.45	1.4
+1.50	*a* 6.3 *L*	+2.20	2.1	+3.60	1.4	+3.85	1.4
+1.30	*a* 6.3 *L*	+2.40	2.1	+3.20	1.4	+3.35	1.4
+1.30	*a* 16.8 *L*	+2.45	2.8	+3.00	2.1	+3.45	1.4
+1.60	*a* 25.2 *L*	+2.35	4.9	+3.00	3.5	+3.30	3.5

L = gelatin liquefied; enzyme in liquefied gelatin; *a* = sugar completely removed.

2. The addition of 0.1% dextrose to the gelatin prior to inoculation fails to prevent the formation of the proteolytic enzyme. During the first 24 hours' growth the organism completely uses up the dextrose and makes some inroad upon the protein constituents of the medium. The only residual evidence of the dextrose is the acid reaction (+ 1.05) and a somewhat smaller amount of ammonia in the medium. The gelatin is completely liquefied and a sterile filtrate of this culture contains the enzyme in an active state.

3. The addition of 0.2% dextrose definitely delays the appearance of the proteolytic enzyme; at the end of 24 hours' growth some dextrose is still present in the gelatin; the effect of utilization of the dextrose upon the metabolism of the organism is clearly shown. The reaction is acid (+ 1.40), and there is no evidence of deaminization. The medium does not liquefy. At the end of 48 hours' incubation the dextrose is found to be completely used up; there is evidence of proteolytic activity at this time, shown not only by the complete liquefaction of the medium, but by a sudden increase in the formation of ammonia as well. In spite of the acid reaction of the medium, there appears to be no delay in the development of the proteolytic enzyme as soon as the dextrose is exhausted.

4. The addition of 0.3% dextrose to the gelatin causes a greater delay in proteolysis. Not until the third day of growth does the dextrose finally disappear. The effect of the dextrose upon the metabolism of the organism during this interval is very definite. As soon as the dextrose is exhausted, formation of ammonia begins immediately, and in spite of the very considerable degree of acidity the liquefaction of the medium is complete within a very few hours. A sterile Berkefeld filtrate of the culture on the third day liquefies gelatin energetically.

5. The addition of 0.4% dextrose or more, definitely prevents proteolysis. The reaction remains acid and even prolonged incubation of the cultures results neither in liquefaction nor increase in ammonia. The accumulation of acid products kills the bacteria.

This experiment shows that small amounts of dextrose, up to a maximum of 0.3%, progressively delay proteolysis and the appearance of the soluble proteolytic enzyme. Both proteolysis and the appearance of the enzyme follow immediately after the dextrose is exhausted. A considerable degree of acidity, the result of the fermentation of the dextrose, appears to be without noteworthy influence upon the production and rate of action of the enzyme.

DISCUSSION

The experiments show that 0.1, 0.2 and 0.3% dextrose delay but do not prevent liquefaction of gelatin by B. proteus. The reaction becomes acid during the exhaustion of the sugar, and the formation of ammonia (deaminization) is restricted until the carbohydrate has disappeared. The reaction then becomes progressively less acid, formation of ammonia increases steadily, and the medium is rapidly liquefied. This experiment also furnishes evidence of the influence of utilizable carbohydrate upon the formation of the enzyme. It shows clearly that the formation of acid, which is produced incidental to the fermentation of the dextrose, is not in itself the decisive initial factor in preventing the appearance of the enzyme in an active state, because the enzyme may be demonstrated in the 3-day culture in 0.3% dextrose gelatin which is markedly acid in reaction (1.5%); at this time the organism has used up the dextrose and has attacked the protein constituents of the medium. This is indicated by an abrupt increase in the formation of ammonia. B. proteus appears to be unable, under the conditions imposed by this experiment, completely to use up 0.4% dextrose; there is no increase in the formation of ammonia and there is no evidence of proteolytic activity.

SUMMARY

In sugar-free gelatin, proteolysis and enzyme formation proceed rapidly from the start and the reaction becomes progressively alkaline as a result of the accumulation of the basic products of putrefaction. The addition of small amounts of dextrose—up to 0.3%—progressively retards the appearance of the enzyme until the sugar is used up. During this period acid products of fermentation, indicating an energetic action upon dextrose, accumulate rapidly, showing that the organism is attacking the carbohydrate, not the protein. When the sugar is exhausted, the organism is forced to derive its energy from protein constituents, and the enzyme is then formed to bring about the necessary changes in the protein to make it assimilable. Larger amounts of dextrose than 0.3% cannot be completely used up by B. proteus and no proteolytic enzyme is formed under these conditions.

These observations have shown that a soluble proteolytic enzyme is not demonstrable in dextrose gelatin cultures of B. proteus as long as dextrose is present. The enzyme promptly appears in an active state, however, as soon as dextrose can no longer be detected, notwithstanding the very considerable degree of acidity of the medium.

It might be assumed that the enzyme was present, but inactivated by the dextrose in itself and that the exhaustion of the dextrose removed the inhibition, leaving the enzyme free to act. This is not the case; sterile filtrates of liquefied gelatin cultures will liquefy sterile 1% dextrose gelatin as rapidly and extensively as sterile dextrose-free gelatin.

It is apparent from the foregoing discussion that moderate amounts of organic acids, resulting from the fermentation of dextrose by B. proteus, do not prevent the formation of the soluble proteolytic enzyme in gelatin after the dextrose is exhausted; it is equally apparent that dextrose in itself does not prevent the liquefaction of sterile dextrose gelatin by the mature enzymes, yet dextrose in gelatin cultures of B. proteus prevents their liquefaction.

The theory which best explains these various phenomena may be stated thus: The addition of dextrose to gelatin cultures of B. proteus protects the protein constituents of the medium—the bacilli utilize the carbohydrate in preference to the protein for energy requirements. Important direct evidence in favor of this sparing action of dextrose for protein is afforded by the prompt appearance of the proteolytic enzyme, and other indications of proteolysis, when the amount of

dextrose does not exceed 0.3% in the experiments cited. B. proteus does not form its characteristic soluble proteolytic enzyme in the presence of dextrose, under the conditions cited.

The tables show a rough parallelism between the liquefaction of gelatin and an increase in the formation of ammonia when dextrose is not present. It is conceivable that the ammonia which accumulates as the organisms act upon the gelatin might be either a by-product of the activity of the enzyme itself—but this is rather improbable, because other bacteria, as B. typhosus and B. coli, form ammonia in sugar-free media but do not produce soluble proteolytic enzymes—or it may be that the formation of ammonia is independent of the action of the enzyme.

The latter possibility would be in accord with the theory that formation of ammonia in cultures of bacteria is not a manifestation of

TABLE 3

COMPARISON OF THE ENZYME ACTIVITIES IN GELATIN OF STERILE FILTRATES FROM CULTURES OF B. PROTEUS IN PLAIN AND DEXTROSE GELATIN

Days	1				2				3			
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*
Plain	*L*	11.2	11.9	*L*	*L*	11.9	11.9	*L*	*L*	12.6	12.6	*L*
0.1% dextrose	*L*	11.2	11.2	*L*	*L*	11.2	11.2	*L*	*L*	12.6	12.6	*L*
0.2% dextrose	*S*	11.2	11.2	*S*	*L*	11.2	11.2	*S*	*L*	12.6	11.9	*L*
0.3% dextrose	*S*	11.2	11.2	*S*	*S*	11.2	11.2	*S*	*L*	11.9	11.9	*S*
0.4% dextrose	*S*	11.2	11.2	*S*	*S*	11.2	11.2	*S*	*S*	11.2	11.2	*S*
0.5% dextrose	*S*	11.2	11.2	*S*	*S*	11.2	11.2	*S*	*S*	11.2	11.2	*S*
1.0% dextrose	*S*	11.2	11.2	*S*	*S*	11.2	11.2	*S*	*S*	11.2	10.5	*S*

Column *a* = physical state of culture, *L* signifying liquefaction, and *S*, no liquefaction.
Column *b* = ammonia expressed as milligrams per 100 c.c. in tube at start (control).
Column *c* = ammonia expressed as milligrams per 100 c.c. in tube after 5 days' incubation.
Column *d* = physical state of tube after 5 days' incubation.

the changes which take place in the protein constituents of the cultural media prior to their assimilation by micro-organisms; it is essentially an index of the intracellular deaminization of the assimilated protein derivatives, incidental to their transformation into energy chiefly. The extracellular changes which the protein constituents of the media undergo are largely of the nature of hydrolytic cleavages; little or no deaminization takes place during this phase of bacterial metabolism. This is shown qualitatively in the following experiment:

Nine-day cultures of B. proteus in plain gelatin (completely liquefied) and 1% dextrose gelatin (solidified promptly when cooled to 18 C.; no liquefaction), respectively, were filtered through Berkefeld filters to remove all bacteria. The

sterile filtrates were added to sterile plain and 1% dextrose gelatin in the proportion of 1 c.c. of filtrate to 10 c.c. of plain and dextrose gelatin, respectively. Suitable controls were made and the various preparations were incubated at 37 C. for 5 days. Sterility was maintained throughout the entire process.

1. Sterile filtrate of 9-day culture of B. proteus, 1 c.c.—Sterile plain gelatin 10 c.c.

Control: Ammonia at start 12.6 mg. The mixture of filtrate and gelatin solidified promptly at room temperature. After 5 days' incubation at 37 C., ammonia 12.6 mg., the mixture was completely liquefied.

2. Sterile filtrate as above, 1 c.c.—sterile dextrose gelatin, 10 c.c.

Control: Ammonia at start 12.6 mg. The mixture of filtrate and gelatin solidified promptly at room temperature. After 5 days' incubation at 37 C., ammonia 12.6 mg., the mixture was completely liquefied.

DISCUSSION

One cubic centimeter of a sterile filtrate of a plain gelatin culture of B. proteus contains a soluble proteolytic enzyme which will completely liquefy ten times the volume of plain or dextrose gelatin. Dextrose gelatin was

TABLE 3—*Continued*

COMPARISON OF THE ENZYME ACTIVITIES IN GELATIN OF STERILE FILTRATES FROM CULTURES OF B. PROTEUS IN PLAIN AND DEXTROSE GELATIN

4				5				10				16			
a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*
L	15.4	15.4	*L*	*L*	16.8	17.5	*L*	*L*	28.0	28.7	*L*	*L*	15.4	15.4	*L*
L	13.3	13.3	*L*	*L*	14.0	14.0	*L*	*L*	18.2	18.9	*L*	*L*	13.3	12.6	*L*
L	12.6	11.9	*L*	*L*	12.6	12.6	*L*	*L*	14.0	14.0	*L*	*L*	11.9	11.2	*L*
L	11.9	11.9	*L*	*L*	11.9	11.9	*L*	*L*	11.9	11.9	*L*	*L*	11.2	11.2	*L*
S	11.2	11.2	*S*	*S*	11.2	11.9	*S*	*S*	11.2	11.2	*S*	*S*	10.5	10.5	*S*
S	11.2	11.2	*S*	*S*	11.2	11.9	*S*	*S*	11.2	10.5	*S*	*S*	10.5	10.5	*S*
S	11.2	10.5	*S*	*S*	11.2	10.5	*S*	*S*	11.2	10.5	*S*	*S*	10.5	10.5	*S*

Column *a* = physical state of culture, *L* signifying liquefaction, and *S*, no liquefaction.
Column *b* = ammonia expressed as milligrams per 100 c.c. in tube at start (control).
Column *c* = ammonia expressed as milligrams per 100 c.c. in tube after 5 days' incubation.
Column *d* = physical state of tube after 5 days' incubation.

liquefied as extensively as plain gelatin, indicating that dextrose in itself had no appreciable effect upon the activity of the mature enzyme. No increase in ammonia was detectable in these solutions, a circumstance showing that the complete liquefaction of the gelatin by the enzyme in the absence of bacteria is accomplished without any indication of deaminization.

A similar experiment was made, using the sterile filtrate of the dextrose-gelatin culture in place of the sterile filtrate from the plain gelatin culture. It will be remembered that the dextrose-gelatin culture was not liquefied and no active proteolytic enzyme was demonstrable in it. The results were wholly negative; no liquefaction of the gelatin occurred and no increase in ammonia was detected. The analytic details are omitted.

A more comprehensive experiment was made in precisely the same manner, with sterile filtrates from the cultures of B. proteus in plain, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, and 1% dextrose gelatin, shown in Table 2.

Table 3 shows the essential analytic details. Column *a* shows the condition of the filtered culture, *L* signifying liquefaction, *S* no liquefaction. Column *b* indicates the amount of ammonia in the mixture of 1 c.c. filtered culture and 10 c.c. sterile gelatin at the beginning of the experiment, and Column *c* indicates the amount of ammonia after 5 days' incubation at 37 C. Column *d* shows the condition of the preparation after 5 days' exposure to 37 C., *L* signifying permanent liquefaction, and *S*, solidification when cooled to room temperature.

The results are self-explanatory. The table shows clearly that liquefaction of gelatin by sterile solutions containing the soluble proteolytic enzyme of B. proteus is not associated with an increase of ammonia. This is interpreted as indication that hydrolytic cleavage of the protein, shown by the liquefaction of the gelatin, is independent of deaminization. The effect of dextrose upon the formation of the proteolytic enzyme is shown in the cultures containing 0.2%, 0.3%, and more of the sugar. A 48-hour culture of the organism in 0.2% dextrose gelatin was liquefied, but the amount of enzyme in 1 c.c. of the filtrate was insufficient to liquefy 10 c.c. of the gelatin to which it was added. A 72-hour culture, however, contained sufficient enzyme to accomplish this purpose. A similar condition was observed in the 3-day culture in 0.3% dextrose gelatin. Here, again, the enzyme was insufficient in concentration to liquefy 10 c.c. of gelatin. The filtrate of the 4-day culture, however, induced complete liquefaction in ten times the volume of gelatin. With 0.4% dextrose or greater amounts the formation of the soluble proteolytic enzyme in cultures of the organism was definitely prevented.

CONCLUSIONS

The foregoing experiments appear to be sufficiently definite to justify the following statements regarding the formation and activity of the soluble proteolytic enzyme of Bacillus proteus.

Bacillus proteus forms a soluble proteolytic enzyme in plain broth and plain gelatin. The mature enzyme may be obtained in an active state free from bacteria by filtering the culture through sterile Berkefeld filters.

The enzyme appears to be a preparatory enzyme in the sense that it prepares protein for assimilation by the bacteria; it has no demonstrable rôle in the intracellular utilization of the protein by the bacteria.

The liquefaction of gelatin by the bacteria-free enzyme is not accomplished by the liberation of ammonia; deaminization is an independent phenomenon associated with the intracellular utilization of the products of proteolysis by the organisms themselves.

The proteolytic enzyme does not appear in an active state in media containing utilizable carbohydrate; it appears only when protein or protein derivatives are being utilized for energy by B. proteus.

Dextrose added to broth or gelatin cultures of B. proteus prevents the formation of the enzyme; under these conditions the enzyme would appear to be unnecessary for the metabolism of the bacteria, because they are acting chiefly upon the dextrose.

Small amounts of dextrose—up to 0.3% in the experiments cited—prevent the formation of the enzyme until the sugar is exhausted; then the bacteria must utilize protein constituents of the medium for energy and consequently enzyme formation begins promptly.

Larger amounts of dextrose than 0.3%, which cannot be wholly exhausted by the bacteria, permanently prevent the formation of the enzyme. It is probable that the accumulation of products of fermentation of the dextrose, principally organic acids, create environmental conditions which inhibit the metabolism of the organisms and eventually lead to their death.

Dextrose in itself does not materially inhibit the activity of the mature, bacteria-free enzyme; sterile dextrose gelatin is as rapidly and completely liquefied as plain gelatin.

The observations presented here do not preclude the possibility that conditions other than those obtaining in the experiments recorded may influence the formation of the enzyme.